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# ISOZYMIC CHARACTERIZATION OF WILD POPULATIONS OF *Cucurbita pepo*

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ABSTRACT.—Isozyme data were collected from 20 wild populations of *Cucurbita pepo* ssp. *ovifera* var. *ozarkana* recently discovered in Arkansas, Illinois, Kentucky, Louisiana, Missouri, and Oklahoma. Comparison of these data to similar data generated for populations in Texas (ssp. *ovifera* var. *texana*) and Tamaulipas, Mexico (ssp. *fraterna*), Mexican landraces (ssp. *pepo*), and cultivars representing both major genetic lineages (ssp. *ovifera* var. *ovifera* and ssp. *pepo*) revealed a distinct isozyme profile, including the characteristic allele *Idh-2m*, for the non-Texas U.S. populations. About half these populations exhibited signs of limited and, in many cases, recent introgression from cultivars. Genetic similarity and the patterns of variation between ssp. *ovifera* var. *ozarkana* and var. *ovifera* lead us to conclude that ancient populations of the former gave rise to the latter. Populations of ssp. *fraterna* from northeastern Mexico also exhibited a unique isozyme profile with affinities to both major cultivar lineages. None of the wild populations exhibited a particularly close relationship to Mexican landraces of ssp. *pepo*, leading us to hypothesize that progenitor populations of these landraces probably occurred farther south in Mexico and may be extinct. In conclusion, the isozyme data revealed that within the range of wild *C. pepo*, genetic divergence took place long before domestication and over an extensive period of time in at least four disjunct and ecologically distinct regions—the central Mississippi valley/Ozark Plateau, Texas, northeastern Mexico, and central or southern Mexico.

RESUMEN—Se colectaron datos de isozimas de 20 poblaciones silvestres de *C. pepo* ssp. *ovifera* var. *ozarkana* descubiertas recientemente en Arkansas, Illinois, Kentucky, Louisiana, Missouri y Oklahoma. La comparación de estos datos con datos similares generados para poblaciones en Texas (ssp. *ovifera* var. *texana*) y Tamaulipas, México (ssp. *fraterna*), variedades mexicanas cultivadas tradicionalmente (ssp. *pepo*), y cultivares que representan los dos linajes genéticos principales (ssp. *ovifera* var. *ovifera* y ssp. *pepo*) reveló un perfil isozimático distintivo, incluyendo el alelo característico *ldh-2m*, para las poblaciones de los Estados Unidos de Norteamérica fuera de Texas. Alrededor de la mitad de estas poblaciones mostraron señas de una introgresión de cultivares, limitada y en muchos casos reciente. La similitud genética y los patrones de variación entre la ssp. *ovifera* var. *ozarkana* y la var. *ovifera* nos conducen a concluir que las poblaciones antiguas de la primera dieron origen a la segunda. Las poblaciones de la ssp. *fraterna* del noreste de México también mostraron un perfil isozimático peculiar con afinidades a los dos linajes principales de cultivares. Ninguna de las poblaciones silvestres mostró una relación particularmente cercana a las variedades mexicanas tradicionalmente cultivadas de la ssp. *pepo*, llevándonos a la hipótesis que las poblaciones progenitoras de estas variedades probablemente se daban más al sur en México y posiblemente se hayan extinguido. En conclusión, los datos de isozimas revelan que dentro del área de distribución de la *C. pepo* silvestre, la divergencia genética tuvo lugar mucho antes de la domesticación y a lo largo de un extenso período de tiempo en por lo menos cuatro regiones geograficamente separadas y ecologicamente diferentes: la región central del Valle del Mississippi junto con la altiplanicie Ozark, Texas, el noreste de México, y el centro o sur de México.

RESUME.—Des données d'isozymes ont été rassemblées, provenant de 20 populations sauvages de *C. pepo* ssp. *ovifera* var. *ozarkana* récemment découvertes dans l'Arkansas, l'Illinois, le Kentucky, la Louisianne, le Missouri, et l'Oklahoma. Des comparaisons faites entre ces données et celles obtenues sur des populations du Texas (ssp. *ovifera* var. *texana*) et de Tamaulipas, Mexico (ssp. *fraterna*), des races mexicaines (ssp. *pepo*), et des "cultivars" representant les deux lignées génétiques principales (ssp. *ovifera* var. *ovifera* et ssp. *pepo*) ont revélé un profile d'isozymes distinct, comprenant le gène (allèle) charactéristique *Idh-2m*, pour la population non-Texanne des USA. A peu près la moitié de ces groupes exhibe des signes d'introgréssion limitée, et, dans plusieurs cas, récente, de "cultivars." La resemblance génétique et le genre de variation entre ssp. *ovifera* var. *ozarkana* et var. *ovifera* nous entraine à conclure que des populations anciennes du premier sont ancestrales à celles du deuxième. Des groupes de ssp. *fraterna* du Nord-Est du Méxique exhibent aussi un profile d'isozyme unique, exhibant des affinitées avec les deux lignées principales. Aucune des populations sauvages exhibent une parenté particulièrement proche des races de ssp. *pepo* du Méxique, ce qui nous amène a supposer que les populations ancestrales de ces races se trouvaient probablement plus au Sud du Méxique, et sont peut-être éteintes. En conclusion, les données d'isozymes ont révelé qu'au sein de l'étendue de *C. pepo* sauvage, une divergence génétique a pris place longtemps avant la domestication, s'étendant sur une longue période, dans 4 régions non-adjacentes et écologiquement distinctes—la vallée du Mississippi central/région du Plateau Ozark, le Texas, le Nord-Est du Méxique, et le centre ou le Sud du Méxique.

## INTRODUCTION

As one of the earliest New World domesticates, squash (*Cucurbita pepo* L.) has great impact on theories of horticultural origins in North America. A greater understanding of the evolutionary history of this species has led to the suggestion that squash domestication took place independently twice to produce the two major lineages of cultivars, *C. pepo* ssp. *ovifera* (L.) Decker var. *ovifera* (L.) Decker and *C. pepo* ssp. *pepo* (Decker 1988). Subspecies *pepo,* which comprises pumpkins, marrows, Mexican landraces, and a few ornamental gourds, apparently has its origins in Mexico, whereas ssp. *ovifera* var. *ovifera,* which includes scallop and crookneck squashes and most ornamental gourds, appears native to the eastern United States (Decker 1988; Decker-Walters 1990). In the past, examination of wild populations that might have given rise to the cultivar lineages was limited to several populations of *C. pepo* ssp. *ovifera* var. *texana* (Scheele) Decker in Texas and a few morphologically similar populations in Alabama, Arkansas, Illinois, and Missouri (Decker and Wilson 1987). Isozyme analyses indicated that these populations were remnants of ancestral wild *C. pepo* and were not merely cultivar escapes (Decker and Wilson 1987). Consequently, var. *texana* became the likely candidate for the progenitor of the var. *ovifera* cultivars. Although isozyme data were lacking at the time, Decker-Walters (1990) hypothesized that *C. pepo* ssp. *fraterna* (Bailey) Andres, which occurs in northeastern Mexico, was the wild progenitor of the ssp. *pepo* lineage of cultivars. No other wild taxa of *C. pepo* are currently recognized.

Recently, a few populations of ssp. *fraterna* became available for isozyme analysis (cf. Wilson 1989). Additionally, a much greater number of wild populations occurring north of Texas were discovered by various collectors (Smith et al. 1992). Because the solid ivory fruits of these northern populations differed from the striped fruits of var. *texana* and ssp. *fraterna,* we decided to look for other genetic evidence distinguishing these populations. Therefore, we collected isozyme data on these and other populations to clarify further the evolutionary relationships within *C. pepo,* particularly those between wild populations and the two domesticated lineages.

## MATERIALS AND METHODS

For purposes of this study, all wild populations occurring north of Texas were considered unclassified, whereas only those populations in Texas were treated as belonging to *Cucurbita pepo* ssp. *ovifera* var. *texana.*

We surveyed isozymes in 20 wild populations of *C. pepo* occurring in states north and east of Texas, six populations of ssp. *ovifera* var. *texana,* two populations of *fraterna,* four cultivars of ssp. *ovifera* var. *ovifera,* and eight cultivars and Mexican landraces of ssp. *pepo* (Tables 1 and 2; Fig. 1). In some analyses, we considered previously collected isozyme data on six non-Texas U.S. populations (Decker and Wilson 1987), 12 additional populations of ssp. *ovifera* var. *texana* (Decker and Wilson 1987), three populations of ssp. *fraterna* (Wilson 1989), and approximately 200 cultivars and landraces (not including Acorn group cultivars) representing ssp. *ovifera* var. *ovifera* and ssp. *pepo* (Decker 1985, 1986; Decker-Walters et al. 1990; Ignart and Weeden 1984; Wilson 1989). Existing data on the closest extant wild relative of *C. pepo, C. argyrosperma* Huber ssp. *sororia* (Bailey) Merrick and Bates (Decker 1986; Wilson 1989), gave us a broader evolutionary perspective on variation within *C. pepo.*

TABLE 1.—Accession and sampling information on 28 wild populations of *Cucurbita pepo*, including ssp. *fraterna* (FRA), var. *texana* (TEX), and related populations (?).

| Accession[1] | State/ Country | County | River/ Creek | No. inds. samp. | No. plants repr. | No. fruits repr. | Reference Numbers[2] |
|---|---|---|---|---|---|---|---|
| ?AR 801 | AR | Benton | Osage | 10 | ? | 6 | AB 1-6 |
| 802 | AR | Searcy | Buffalo | 11 | 11 | 11 | AB 201 205 217 226 235 239 241 262 278 282 285 |
| 803 | AR | Benton | Spavinaw | 10 | ? | 10 | AB 16 18 23 25 27 28 31-34 |
| 804 | AR | Benton | Little Sugar | 8 | ? | 8 | AB 7 9 10 55 57 72 74 75 |
| 818 | AR | Searcy | Buffalo | 9 | 1 | 9 | AB 183-187 189-192 |
| 819 | AR | Izard | White | 9 | 5 | 9 | AB 288-290 294 295 321 322 329 330 |
| 820 | AR | Independence | White | 8 | 2 | 8 | AB 362 364-366 373-375 378 |
| ?IL 812 | IL | Jersey | Illinois | 10 | ? | 2 | AB 108 109 |
| ?KY 813 | KY | Powell | Red | 9 | 1 | 1 | AB 110 |
| ?LA 811 | LA | Tenasas | Mississippi | 8 | 1 | 1 | AB 107 |
| 814 | LA | Bossier | Red | 13 | ? | 11 | AB 387-390 392 394 395 398-401 |
| ?MO 808 | MO | Christian | Wilson | 9 | ? | 3 | AB 80-82 |
| 809 | MO | Greene | James | 10 | ? | 2 | AB 83 84 |
| 810 | MO | Taney | Swan | 10 | ? | 10 | AB 86 90-98 |
| 815 | MO | Wright | Gasconade | 9 | 3 | 9 | AB 118 119 121 125 126 128 135-137 |
| 816 | MO | Wright | Gasconade | 13 | 2+ | 10 | AB 152 154-162 |
| 822 | MO | Ozark | Bryant | 14 | 1 | 7 | AB 111-117 |
| ?OK 805 | OK | Mayes | Verdigris | 9 | 1 | 1 | AB 13 |
| 806 | OK | Cherokee | Illinois | 12 | ? | 4 | AB 51-54 |
| 807 | OK | Adair | Illinois | 8 | ? | 2 | AB 99 100 |
| FRA 750 | MEX | Tamaulipas | — | 10 | ? | 8 | P.I. 532355 |
| 751 | MEX | Tamaulipas | — | 9 | ? | 5 | P.I. 532354 |
| TEX 747 | TX | Gonzales | Guadalupe | 11 | 11? | 11? | P.I. 285213 |
| 779 | TX | San Patricio | Aransas | 8 | ? | 8? | TEX 1 |
| 780 | TX | Refugio | Medio | 8 | ? | 4? | TEX 2 |
| 781 | TX | Goliad | San Antonio | 8 | ? | 7? | TEX 3 |
| 782 | TX | Gonzales | Guadalupe | 8 | ? | 8? | TEX 4 |
| 783 | TX | Fayette | Colorado | 8 | ? | 8? | TEX 5 |

[1]Accession numbers are the collection numbers of D. Decker-Walters and T. Walters.

[2]AB numbers are curation numbers assigned to fruits deposited in the Archaeobiology Laboratory at the National Museum of Natural History. P.I. numbers are U.S.D.A. Plant Introduction numbers. TEX numbers are from Decker and Wilson (1987).

TABLE 2.—Accession and sampling information on eight cultivars and four Mexican landraces of *Cucurbita pepo*.

| Taxon | Accession[1] | Cultivar/ Landrace | Source[2] | No. individuals sampled |
|---|---|---|---|---|
| ssp. *ovifera* | | | | |
| var. *ovifera* | FMA 467 | 'Mandan' | — | 11 |
| | OEN 538 | 'Nest Egg' | Nichols Garden Nursery | 8 |
| | OFS 774 | 'Flat Striped' | Stokes | 8 |
| | OPS 763 | 'Striped Pear' | J.L. Hudson | 8 |
| ssp. *pepo* | OBO 775 | 'Orange Ball' | Stokes | 9 |
| | OWO 765 | 'Orange Warted' | J.L. Hudson | 10 |
| | PCO 759 | 'Connecticut Field' | J.L. Hudson | 8 |
| | PSU 772 | 'Small Sugar' | Stokes | 8 |
| | XGU 777 | Mexican landrace | P.I. 512190 | 8 |
| | XHU 543 | Mexican landrace | Stokes | 8 |
| | XQU 778 | Mexican landrace | P.I. 512192 | 8 |
| | XYU 468 | Mexican landrace | — | 5 |

[1]Accession numbers are the collection numbers of D. Decker-Walters and T. Walters.

[2]All seed was obtained from seed companies or the U.S.D.A., except for FMA 467, which was contributed by G. Drowns, and XYU 468, which is $F_1$ seed of a self of P.I. 438699 created by T. Andres.

The following northern populations showed morphological signs (e.g., non-bitterness, large seeds, thick peduncles) of hybridization with cultivars: ?AR802, ?AR819, ?KY813, ?LO814, ?MO816 (Smith et al. 1992; Cowan and Smith, this volume). Morphological evidence of introgression was also discovered in wild populations from Illinois and Alabama (Decker and Wilson 1987).

We examined isozymes in five to 13 individuals per accession (Tables 1 and 2). Enzymes were extracted from 3- to 5-day-old cotyledons as described in Decker-Walters et al. (1990). We employed two buffer systems to resolve the following eight enzyme systems: on a citric acid/morpholine (pH = 6.7) system, isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKD); and on the Poulik system (system #1 of Kirkpatrick et al. 1985), aspartate aminotransferase (AAT), glycerate dehydrogenase (G2D), glucose-6-phosphate isomerase (GPI), and leucine aminopeptidase (LAP). Recipes and additional details on the electrophoretic methodology are described elsewhere (Decker-Walters et al. 1990; Kirkpatrick et al. 1985).

Genetic interpretation was based on the nature of the banding patterns and on progeny segregation results from a cross of *C. pepo* ssp. *pepo* and ssp. *ovifera* var. *texana* performed by Kirkpatrick et al. (1985). As suggested by Kirkpatrick et al. (1985), the large number of loci detected for some enzymes (e.g., PGM) probably reflects gene duplication of a polyploid nature for the genus *Cucurbita*. However, progeny segregation analysis confirmed that species of this genus behave genetically as diploids (Kirkpatrick et al. 1985).

We resolved allelic variation at 18 enzyme loci: *Aat-1, Aat-3, Aat-4, G2d-1, G2d-2, Gpi-2, Gpi-3, Idh-1, Idh-2, Idh-3, Lap-1, Mdh-1, Mdh-2, Mdh-3, Pgm-1, Pgm-5,*

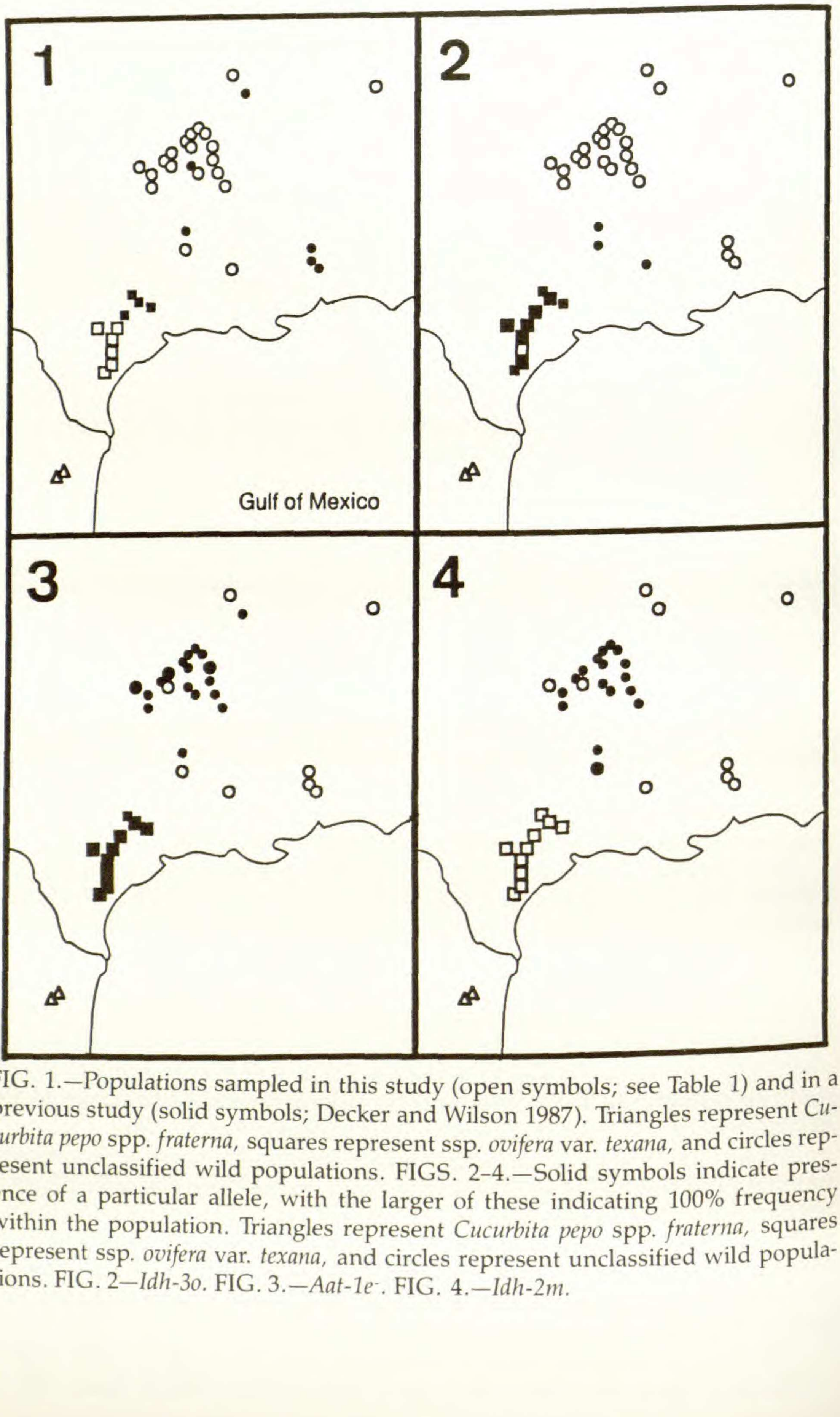


FIG. 1.—Populations sampled in this study (open symbols; see Table 1) and in a previous study (solid symbols; Decker and Wilson 1987). Triangles represent *Cucurbita pepo* spp. *fraterna,* squares represent ssp. *ovifera* var. *texana,* and circles represent unclassified wild populations. FIGS. 2-4.—Solid symbols indicate presence of a particular allele, with the larger of these indicating 100% frequency within the population. Triangles represent *Cucurbita pepo* spp. *fraterna,* squares represent ssp. *ovifera* var. *texana,* and circles represent unclassified wild populations. FIG. 2—*Idh-3o.* FIG. 3.—*Aat-1e*$^{-}$. FIG. 4.—*Idh-2m.*

*Pgm-6, Skd-1. Pgm-6* is equivalent to *Pgm-2* of previous reports (e.g., Decker 1985; Wilson 1989). We designated alleles in accordance with Kirkpatrick et al. (1985) and Decker-Walters et al. (1990) with the following exceptions: *Idh-1g* of previous studies has been renamed *Idh-1e* because results herein indicate that the enzyme product of this allele does not comigrate with that of *Idh-2g* on the gel. Note that Wilson (1989) interprets our *Idh-2m* (which we proposed in Decker-Walters et al. 1990) as a null or inactive variant (his *Idh-2z*). Because either interpretation is equally plausible based on currently known banding patterns, we have chosen to follow Decker-Walters et al. (1990) to be consistent with our previous work, while at the same time recognizing that the null interpretation may be correct. Our null variants are denoted with a superscripted "n" (e.g., *Lap-1f*$^{n}$).

The ability to detect first-generation hybridization is one of the advantages of isozyme data. Heterozygous conditions are clearly visible and when an individual is heterozygous for alleles typically not found in that taxon, it is often possible to determine the taxon that contributed the "foreign" pollen. The opportunity to document infraspecific hybridization is greater in *Cucurbita pepo* than in most species because there are at least two genetically distinct but interfertile lineages: ssp *pepo* and ssp. *ovifera* (Decker 1988). Consequently, we looked for signs of first-generation hybridization as well as longer histories of introgression in the isozyme data on the wild populations. First-generation hybridization could be postulated for an individual exhibiting a larger than normal percentage of heterozygous loci at which one of the alleles per locus rarely or never occurred in other members of the same population and/or taxon. Introgression beyond the first generation was suspected when one of these apparently foreign alleles was found in the homozygous state.

Other data analyses also focused on describing wild populations north of Texas and on comparing these populations to infraspecific taxa of *C. pepo* and to *C. argyrosperma* ssp. *sororia*. We mapped alleles in wild populations of *C. pepo* to look for geographical trends. Genetic affinities within *C. pepo* were illustrated via the plotted results of principal component analysis (PCA), which was performed using the Statistical Analysis System (SAS Institute Inc. 1985). Following Nei (1972), as in previous studies, we calculated genetic identity values within and among taxa using the data presented herein, except for data on *C. argyrosperma* ssp. *sororia*, which came from Decker (1986).

Drawing on all available sources of isozyme data (see above), we attempted to characterize each taxon, including the unclassified populations occurring north of Texas, by way of an isozyme profile. We focused only on those variable loci for which abundant data were available: *Aat-1, Gpi-3, Idh-1, Idh-2, Idh-3, Mdh-2, Mdh-3, Pgm-6*. Alleles found rarely or as apparent introgressants in a taxon were not included in that taxon's isozyme profile. Introgressants were presumed based on heterozygous conditions such as those described in general terms, above, and more specifically, below.

## RESULTS

The frequencies of 40 alleles at 15 variable enzyme loci are presented in Table 3. All accessions were fixed for *Gpi-2l, Mdh-1c,* and *Pgm-1a*. Many other alleles were widespread across accessions and therefore uninformative (e.g., *G2d-1n, Idh-1e, Idh-2g, Lap-1f, Mdh-2i, Pgm-6v*).

TABLE 3.—Frequencies (expressed as percentages) of 40 alleles at 15 enzyme loci for 28 wild populations, eight cultivars, and four Mexican landraces of *Cucurbita pepo*.

| Locus | Aat-1 | | | Aat-3 | | Aat-4 | | | G2d-1 | | | G2d-2 | | Gpi-3 | | Idh-1 | | Idh-2 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Allele | *b* | *d* | *e* | *g* | *j* | *r* | *u* | *v* | *i* | *k* | *n* | *v* | $x^n$ | *o* | *u* | *b* | *e* | *g* | *m* |
| Wild populations: | | | | | | | | | | | | | | | | | | | |
| ?AR801 | 25 | 75 | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 |
| ?AR802 | 0 | 45 | 55 | 0 | 100 | 86 | 14 | 0 | 5 | 0 | 95 | 100 | 0 | 0 | 100 | 0 | 100 | 55 | 45 |
| ?AR803 | 0 | 5 | 95 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 75 | 25 |
| ?AR804 | 0 | 0 | 100 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 94 | 6 |
| ?AR818 | 0 | 89 | 11 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 89 | 11 |
| ?AR819 | 0 | 39 | 61 | 0 | 100 | 100 | 0 | 0 | 11 | 0 | 89 | 100 | 0 | 0 | 100 | 0 | 100 | 33 | 67 |
| ?AR820 | 0 | 50 | 50 | 0 | 100 | 63 | 12 | 25 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 81 | 19 |
| ?IL812 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 |
| ?KY813 | 0 | 100 | 0 | 0 | 100 | 100 | 0 | 0 | 39 | 0 | 61 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 |
| ?LA811 | 0 | 100 | 0 | 0 | 100 | 88 | 12 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 |
| ?LA814 | 0 | 100 | 0 | 0 | 100 | 100 | 0 | 0 | 27 | 0 | 73 | 100 | 0 | 0 | 100 | 23 | 77 | 0 | 100 |
| ?MO808 | 0 | 44 | 56 | 0 | 100 | 89 | 11 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 67 | 33 |
| ?MO809 | 0 | 50 | 50 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 50 | 50 |
| ?MO810 | 0 | 40 | 60 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 80 | 20 |
| ?MO815 | 0 | 22 | 78 | 22 | 78 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 56 | 44 |
| ?MO816 | 15 | 58 | 27 | 8 | 92 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 65 | 35 |
| ?MO822 | 0 | 0 | 100 | 4 | 96 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 4 | 96 | 18 | 82 |
| ?OK805 | 0 | 0 | 100 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 |
| ?OK806 | 0 | 63 | 37 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 96 | 4 |
| ?OK807 | 0 | 44 | 56 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 88 | 12 |
| FRA750 | 0 | 100 | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 15 | 85 | 100 | 0 | 35 | 65 | 20 | 80 | 100 | 0 |
| FRA751 | 0 | 100 | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 |
| TEX747 | 0 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 |
| TEX779 | 0 | 0 | 100 | 0 | 100 | 88 | 12 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 |
| TEX780 | 0 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 |
| TEX781 | 0 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 |
| TEX782 | 0 | 0 | 100 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 |
| TEX783 | 0 | 0 | 100 | 0 | 100 | 56 | 44 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 |
| var. *ovifera:* | | | | | | | | | | | | | | | | | | | |
| FMA467 | 50 | 50 | 0 | 0 | 100 | 50 | 50 | 0 | 36 | 0 | 64 | 100 | 0 | 0 | 100 | 28 | 72 | 100 | 0 |
| OEN538 | 12 | 88 | 0 | 69 | 31 | 100 | 0 | 0 | 0 | 0 | 100 | 94 | 6 | 25 | 75 | 6 | 94 | 100 | 0 |
| OFS774 | 0 | 100 | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 6 | 94 | 0 | 100 | 100 | 0 |
| OPS763 | 0 | 100 | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 |
| ssp. *pepo:* | | | | | | | | | | | | | | | | | | | |
| OBO775 | 22 | 78 | 0 | 33 | 67 | 100 | 0 | 0 | 6 | 0 | 94 | 100 | 0 | 17 | 83 | 17 | 83 | 100 | 0 |
| OWO765 | 55 | 45 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 100 | 0 | 5 | 95 | 100 | 0 |
| PCO759 | 69 | 31 | 0 | 100 | 0 | 100 | 0 | 0 | 6 | 0 | 94 | 94 | 6 | 94 | 6 | 94 | 6 | 100 | 0 |
| PSU772 | 75 | 13 | 12 | 100 | 0 | 94 | 6 | 0 | 0 | 0 | 100 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 |
| XGU777 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 100 | 0 | 94 | 6 | 100 | 0 |
| XHU543 | 100 | 0 | 0 | 97 | 3 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 100 | 0 | 83 | 17 | 100 | 0 |
| XQU778 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 100 | 0 | 25 | 75 | 100 | 0 |
| XYU468 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 |

| Idh-3 | | Lap-1 | | | | Mdh-2 | | Mdh-3 | | | Pgm-5 | | Pgm-6 | | Skd-1 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *m* | *o* | *e* | *f* | *f*$^{n}$ | *g* | *e* | *i* | *m* | *q* | *u* | *n* | *o* | *s* | *v* | *g* | *h* | *l* | *k* | *m* | *p* |
| 100 | 0 | 0 | 100 | 0 | 0 | 85 | 15 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 18 | 82 | 14 | 86 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 5 | 0 | 95 | 0 |
| 100 | 0 | 0 | 85 | 0 | 15 | 25 | 75 | 5 | 95 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 37 | 63 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 37 | 0 | 63 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 28 | 72 | 11 | 89 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 |
| 100 | 0 | 0 | 89 | 0 | 11 | 33 | 67 | 61 | 39 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 72 | 28 |
| 100 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 25 | 75 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 37 | 0 | 63 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 20 | 80 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 56 | 44 | 56 | 0 | 44 | 0 | 100 | 50 | 50 | 0 | 0 | 100 | 0 | 0 | 0 |
| 44 | 56 | 0 | 56 | 44 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 6 | 0 | 94 | 0 |
| 85 | 15 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 44 | 56 | 11 | 89 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 22 | 0 | 78 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 50 | 50 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 75 | 0 | 25 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 30 | 0 | 70 | 0 |
| 100 | 0 | 0 | 83 | 17 | 0 | 17 | 83 | 0 | 72 | 28 | 0 | 100 | 17 | 83 | 0 | 0 | 0 | 0 | 100 | 0 |
| 100 | 0 | 8 | 92 | 0 | 0 | 4 | 96 | 0 | 77 | 23 | 0 | 100 | 0 | 100 | 4 | 0 | 0 | 0 | 96 | 0 |
| 100 | 0 | 0 | 96 | 0 | 4 | 100 | 0 | 82 | 18 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 4 | 0 | 96 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 4 | 96 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 6 | 0 | 0 | 0 | 94 | 0 |
| 100 | 0 | 0 | 40 | 0 | 60 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 90 | 10 | 0 | 0 | 0 | 100 | 0 | 0 |
| 100 | 0 | 0 | 61 | 0 | 39 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 6 | 94 | 0 | 0 | 0 | 100 | 0 | 0 |
| 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 0 |
| 12 | 88 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 |
| 0 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 |
| 0 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 |
| 0 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 9 | 91 | 0 | 100 | 0 | 0 | 0 | 6 | 94 | 0 |
| 100 | 0 | 19 | 62 | 0 | 19 | 44 | 56 | 38 | 6 | 56 | 0 | 100 | 14 | 86 | 50 | 0 | 0 | 0 | 50 | 0 |
| 100 | 0 | 0 | 81 | 0 | 19 | 0 | 100 | 37 | 44 | 19 | 0 | 100 | 6 | 94 | 0 | 0 | 19 | 0 | 81 | 0 |
| 100 | 0 | 0 | 88 | 0 | 12 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 56 | 0 | 44 | 0 |
| 100 | 0 | 0 | 17 | 0 | 83 | 0 | 100 | 89 | 11 | 0 | 0 | 100 | 94 | 6 | 100 | 0 | 0 | 0 | 0 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 25 | 5 | 70 | 10 | 90 | 100 | 0 | 75 | 0 | 0 | 75 | 0 | 0 |
| 100 | 0 | 19 | 6 | 0 | 75 | 0 | 100 | 0 | 25 | 75 | 0 | 100 | 81 | 19 | 69 | 0 | 13 | 12 | 6 | 0 |
| 100 | 0 | 50 | 0 | 0 | 50 | 19 | 81 | 0 | 0 | 100 | 0 | 100 | 75 | 25 | 38 | 0 | 6 | 37 | 19 | 0 |
| 100 | 0 | 0 | 50 | 0 | 50 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 | 75 | 0 | 0 | 0 | 25 | 0 |
| 100 | 0 | 73 | 0 | 0 | 27 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 83 | 17 | 22 | 11 | 0 | 0 | 67 | 0 |
| 100 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 0 | 94 | 0 | 0 | 0 | 6 | 0 |
| 100 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 0 |

Some alleles indicated a genetic link between populations in Texas and those farther north. As noted in an earlier study (Decker and Wilson 1987), *Idh-3o* is an allele that characterizes var. *texana* (Table 3; Fig. 2). It has never been found in any cultivar or landrace of var. *ovifera* or ssp. *pepo* (Table 3; Decker 1986; Wilson 1989). However, this allele was detected in both populations from Louisiana (Table 3) and in a population from southwestern Arkansas (Fig. 2; Decker and Wilson 1987). The allele *Aat-1e*⁻ exhibited a similar pattern; it characterizes populations of var. *texana*, is rare in domesticated material (Decker 1986), and was found in several of the populations north of Texas (Fig. 3). In fact, three non-Texas populations (?AR801, ?MO822, ?OK805) were fixed for *Aat-1e*⁻ (Table 3; Fig. 3). Another allele linking Texas populations to those farther north is *Aat-4u* (Table 3). We found this allele in all but one Texas population, in five populations to the north (?AR802, ?AR820, ?IL812, ?LA811, ?MO808), and in two cultivars (FMA467, PSU772). However, since only cultivars in this study have been tested for variation at *Aat-4*, the distribution of allele "*u*" in domesticated material in general cannot yet be determined.

Within *C. pepo*, a few alleles, including *Aat-4v*⁻, *Idh-2m*, and *Lap-1f*ⁿ, were unique to populations north of Texas (Table 3). Of these, only *Idh-2m* was widespread, characterizing populations in Arkansas, Missouri, and northwestern Louisiana (Fig. 4). This allele, which was interpreted by Wilson (1989) as a null or inactive variant, appears to occur as a dominant allele in *C. argyrosperma* as well (Decker 1986; Decker-Walters et al. 1990; Wilson 1989). If introgression from *C. argyrosperma* accounted for the widespread presence of *Idh-2m* in populations of *C. pepo*, then we should have found other alleles characteristic of *C. argyrosperma* (e.g., *Gpi-3o, Lap-1c, Skd-1s;* Decker-Walters et al. 1990) in at least a few of these populations; we did not (Table 3). Remaining possible explanations are that *Idh-2m* was either present in the shared ancestor of these two species or had separate origins in these species or their ancestors. The latter interpretation would be supported if *Idh-2m* were a null allele. In either case, these scenarios indicate that many of the wild populations north of Texas could not be cultivar escapes since this allele has not been found in cultivars of *C. pepo*.

Table 3 quickly reveals that the unclassified northern populations exhibited greater genetic affinities to ssp. *ovifera* and ssp. *fraterna* than to ssp. *pepo*. Inasmuch as previous isozyme, morphological, and DNA studies (Decker 1986, 1988; Wilson et al. 1992) already indicated that ssp. *pepo* has followed a distinct evolutionary path, alleles that characterized ssp. *pepo* and occurred in few wild populations, usually as one or two heterozygous individuals, were assessed as cultivar introgressants in those populations; these include *Aat-1b, Aat-3g, Idh-1b, Lap-1e, Mdh-3u, Pgm-6s,* and *Skd-1g* (Table 3). Using these alleles as markers, we detected first-generation hybridization resulting from cultivar pollen on wild stigmas in ?AR803, ?LA814, ?MO822, and ?OK807. For example, the genetic configuration of one individual in ?MO822 included *Aat-3gj, Idh-1be*⁻, *Lap-1fg,* and *Skdj-1im,* whereas the remaining individuals, including another from the same fruit, were homozygous at these loci for the alleles commonly found in the northern populations (*Aat-3jj, Idh-1e*⁻*e*⁻, *Lap-1ff, Skd-1mm*). In a previous study (Decker and Wilson 1987), similar evidence, in the form of heterozygous progeny, was discovered for populations in Alabama, Illinois, and even Texas. In the more recently surveyed populations, a longer history of gene flow, as suggested by a few individuals homozygous for alleles listed above, was evident in ?AR801, ?AR819, ?MO815, and ?MO816. In

TABLE 4.—Isozyme profiles for taxa of *Cucurbita pepo* and *C. argyrosperma* spp. *sororia*. Data from other sources (e.g., Decker 1986; Wilson 1989) were considered also. Acorn cultivars were not included in *C. pepo* spp. *ovifera* var. *ovifera*, whereas ssp. *pepo* was represented by Mexican landraces only.

| | Aat-1 | Gpi-3 | Idh-1 | Idh-2 | Idh-3 | Mdh-2 | Mdh-3 | Pgm-6 |
|---|---|---|---|---|---|---|---|---|
| *C. pepo* | | | | | | | | |
| var. *ovifera* | d | u | e⁻ | g | m | e i | m q u | v |
| northern populations | d e⁻ | u | e⁻ | g m | m | e i | m q | v |
| var. *texana* | e⁻ | u | e⁻ | g | m o | i | q | v |
| ssp. *fraterna* | d | o u | b e⁻ | g | m | i | q | s v |
| ssp. *pepo* | b | o | b e⁻ | g | m | i | u | s v |
| *C. argyrosperma* | | | | | | | | |
| ssp. *sororia* | e⁻ | o | e⁻ | g m | m | i | q | v |

TABLE 5.—Nei's (1972) genetic identity values (percentages) calculated for *C. pepo* ssp. *ovifera* var. *ovifera*, ssp. *ovifera* var. *texana*, ssp. *fraterna*, spp. *pepo*, and unclassified northern populations.

| Taxon | O | ? | T | F | P |
|---|---|---|---|---|---|
| var. *ovifera* (O) | 89 | | | | |
| northern populations (?) | 89 | 93 | | | |
| var. *texana* (T) | 79 | 86 | 92 | | |
| ssp. *fraterna* (F) | 84 | 85 | 76 | 95 | |
| ssp. *pepo* (P) | 68 | 64 | 56 | 71 | 87 |

?MO816, for example, one individual was homozygous for *Lap-1ee* and *Mdh-3uu* and heterozygous for *Aat-1bd*. In each of these cases the putative introgressant alleles occurred within the population at a frequency less than 30% (Table 3) except for ?KY813, which had high frequencies of *Mdh-3u* (44%) and *Pgm-6s* (50%), suggesting that this fruit may have come from a feral domesticated plant.

One allele that was surprisingly missing from northern populations was *Gpi-3o*. This allele, which is dominant in ssp. *pepo* cultivars (Table 4; Decker 1986, 1988), would be expected to occur in wild populations if cultivar introgression were common, unless selection in the wild acted to remove this allele, probably as the result of linkage to a morphological or ecological character.

One reason why foreign alleles were easily recognizable is because most of the populations north of Texas possessed a unique and largely coherent genetic profile (Table 4). This profile is most like that of the ssp. *ovifera* var. *ovifera* lineage of cultivars, with some similarity to var. *texana* and ssp. *fraterna* as well (Table 4).

These profiles along with the genetic identity values (Table 5) suggest relationships among the other taxa. The taxon most genetically similar to the ssp. *pepo* lineage is ssp. *fraterna* (Table 5). However, there are significant differences between these taxa at *Aat-1* and *Mdh-3* (Table 4), and ssp. *fraterna* exhibits larger genetic identity values with ssp. *ovifera* than it does with ssp. *pepo* (Table 5).

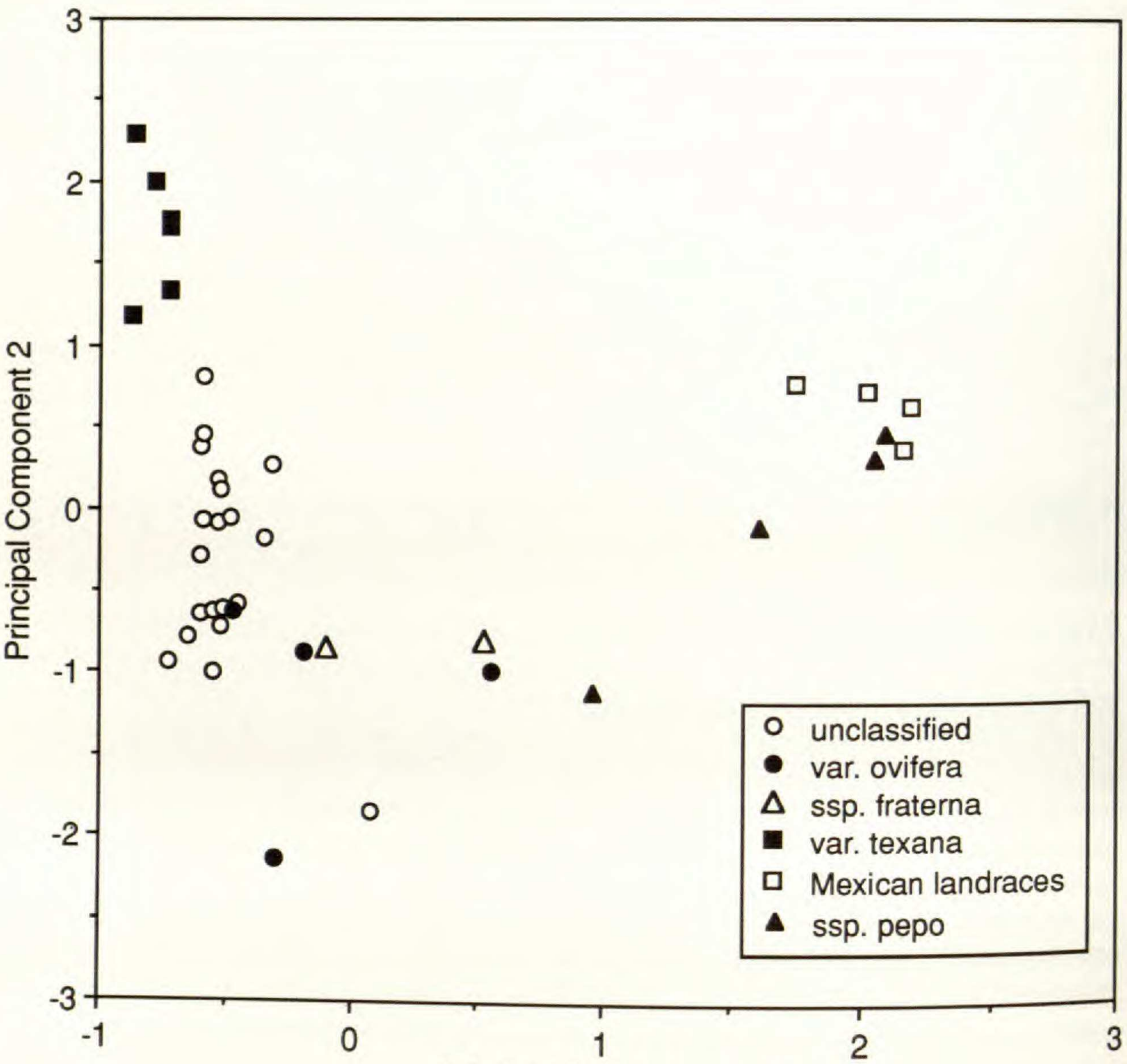


FIG. 5.—Plot of the first two principal components in the analysis of 20 unclassified populations of *Cucurbita pepo,* four cultivars of ssp. *ovifera* var. *ovifera,* two populations of ssp. *fraterna,* six populations of ssp. *ovifera* var. *texana,* and four Mexican landraces and four cultivars of ssp. *pepo*.

Taxon relationships are also revealed in the plotted results of the principal component analyses (Figs. 5 and 6). In the first analysis (Fig. 5), which included all accessions, the first component (PC1), accounting for 47% of the total variation, served to distinguish cultivars and Mexican landraces of ssp. *pepo*. Among members of ssp. *pepo* only 'Orange Ball' had a relatively low value for PC1 (0.963), placing it near 'Nest Egg' (PC1 = 0.560) and one of the populations of ssp. *fraterna* (PC1 = 0.527). Populations of ssp. *ovifera* var. *texana* were pulled away from other accessions by having high values for PC2, which accounted for 13% of the total variation. The unclassified northern populations clustered more or less together, except for the accession from Kentucky (PC2 = –1.839). 'Striped Pear' also had a low value for PC2 (–2.143), whereas 'Mandan' clustered with the northern populations and 'Flat Striped' occurred very close to one of the populations of ssp. *fraterna*.

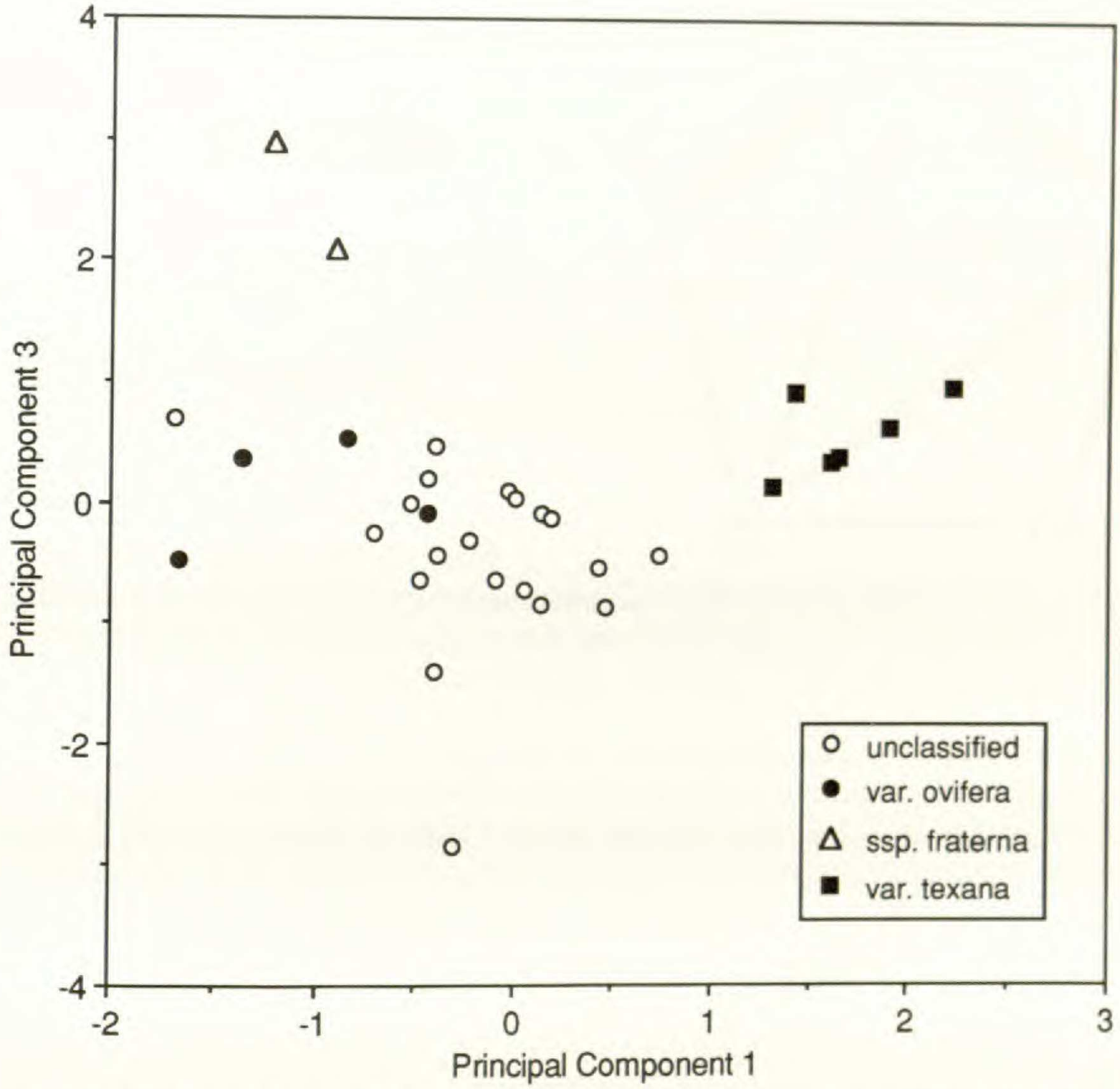


FIG. 6.—Plot of the first and third principal components in the analysis of 20 unclassified populations of *Cucurbita pepo,* four cultivars of ssp. *ovifera* var. *ovifera,* two populations of ssp. *fraterna,* and six populations of ssp. *ovifera* var. *texana.*

To further clarify the genetic affinities of domesticated ssp. *ovifera,* principal component analysis was performed after excluding domesticated ssp. *pepo* (Fig. 6). Along PC1, accounting for 30% of the total variation in this analysis, populations of ssp. *ovifera* var. *texana* were once again distinguished from remaining accessions. No distinct groupings were revealed by PC2 (not shown), which accounted for 20% of the total variation. Populations of ssp. *fraterna* had high values for PC3, which accounted for 14% of the total variation (Fig. 6). Once again, 'Mandan' clustered with the bulk of northern populations. Lower PC1 values were seen in 'Flat Striped' (PC1 = -0.850), 'Nest Egg' (-1.362), 'Striped Pear' (-1.663), and the fruit from Kentucky (-1.689). ?MO822 displayed the lowest value for PC3 (Fig. 6).

In summary, the principal component analyses revealed the following genetic affinity groupings in descending order of distinctiveness: ssp. *pepo,* ssp. *ovifera* var. *texana,* and ssp. *fraterna.* Although, as a group, cultivars of ssp. *ovifera* var. *ovifera* did not appear to cluster randomly among the northern populations, neither were they completely separated from these populations as a distinct grouping (Figs. 5 and 6).

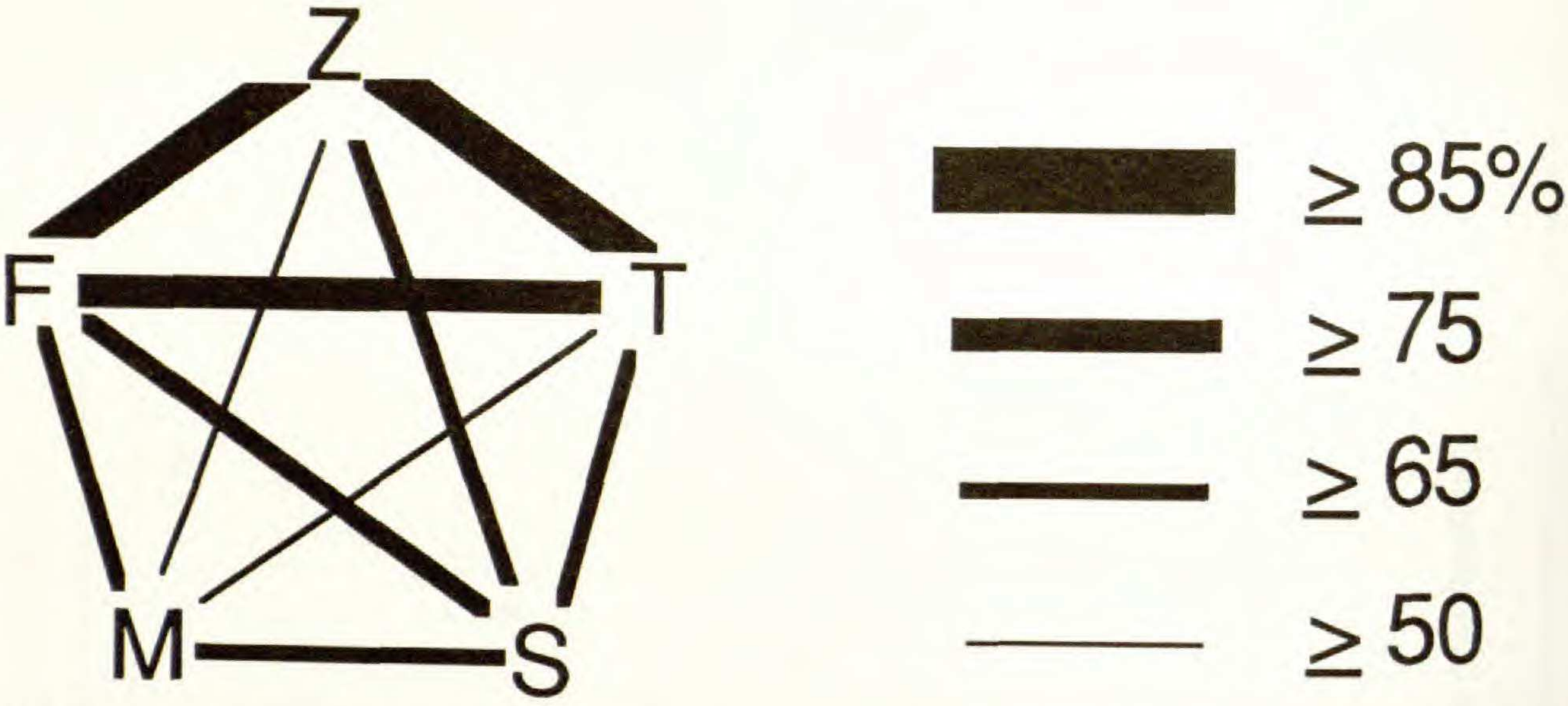


FIG. 7.—Nei's (1972) genetic identity values among *Cucurbita pepo* ssp. *fraterna* (F), Mexican landraces of ssp. *pepo* (M), ssp. *ovifera* var. *texana* (T), *C. argyrosperma* ssp. *sororia* (S), and unclassified northern populations (Z).

Previous phylogenetic analysis (Decker-Walters et al. 1990) indicates that the isozyme profile of *C. argyrosperma* ssp. *sororia* (Table 4) contains mostly symplesiomorphic (having originated in an earlier ancestor) alleles that are shared with *C. pepo*. In fact, the only uncertainties in this regard are *Idh-2m,* which was discussed earlier, and alleles at *Aat-1*. Consequently, differences between *C. argyrosperma* ssp. *sororia* and various groups of *C. pepo* are due primarily to derived character states in *C. pepo* (Table 4). Neither the profiles nor the genetic identity values (Fig. 7) suggest that *C. argyrosperma* ssp. *sororia* exhibits a closer relationship to one *C. pepo* taxon than it does to the other *C. pepo* taxa. In other words, divergence of each *C. pepo* taxon from the common ancestor of *C. argyrosperma* spp. *sororia* and *C. pepo* has occurred at very similar rates. Interestingly, genetic divergence between Mexican landraces of ssp. *pepo* and wild U.S. populations is greater than that between *C. argyrosperma* ssp. *sororia* and taxa of *C. pepo* (Fig. 7).

## DISCUSSION

*The status of northern populations.*—As a group, wild populations of *C. pepo* north of Texas possessed a distinct though variable genetic profile, which usually included *Idh-2m* and the ssp. *ovifera* var. *texana* allele, *Aat-1e*⁻. Louisiana and Arkansas populations close to Texas also exhibited an allele otherwise restricted to var. *texana, Idh-3o*. The overall isozyme profile and these alleles in particular are evidence that populations north of Texas developed their own genetic identity, probably over a long period of relative isolation.

The northern populations display close isozyme affinities to cultivars of var. *ovifera,* var. *texana,* and ssp. *fraterna*. However, morphologically and ecologically ssp. *fraterna* is distinct from the remaining wild populations (Andres 1987). For example, mature fruits of ssp. *fraterna* turn yellow-orange whereas those of var. *texana* and the more northern populations do not. Also, ssp. *fraterna* seeds are relatively slow to germinate (within 5–15 days) and the plants are found in upland

habitats as opposed to the riparian habitats characterizing var. *texana* and populations to the north (i.e., Cowan and Smith, this volume). On the bases of fruit characters and ecogeography, we recognize wild populations north of Texas as more closely related to var. *texana* than to ssp. *fraterna*. Furthermore, ecogeographic, fruit structure, and allozyme data indicate that these populations can be circumscribed as a distinct variety of ssp. *ovifera*, as described below. The circumscription of var. *texana* is changed from Decker (1988) to include Texas populations only.

Key to the Varieties of *Cucurbita pepo* ssp. *ovifera*:

1. Fruit almost always bitter, solid ivory or green- and white-striped but never yellow or orange, rind smooth; seed germination within 1–7 days; possessing *Idh-2m* and/or *Idh-3o;* wild.
   2. Fruit typically solid ivory; germination within 1–4 days; possessing *Idh-2m*, may possess *Idh-3o;* Illinois, Missouri, Arkansas, Oklahoma, Louisiana. var. *ozarkana*
   2. Fruit typically green- and white-striped; germination within 3–7 days; possessing *Idh-3o*, lacking *Idh-2m;* Texas. var. *texana*
1. Fruit non-bitter (except for some ornamental gourd cultivars), solid or striped, in a variety of colors including yellow and orange, rind smooth, ribbed, or warted; germination within 3–15 days depending on the cultivar; lacking *Idh-2m* and *Idh-3o;* under cultivation. var. *ovifera*

**Cucurbita pepo** L. ssp. **ovifera** (L.) Decker var. **ozarkana** Decker-Walters, var. nov., OZARK GOURD.—TYPE: USA, Arkansas, Independence County, Nov 6, 1990, *B. Smith & W. Cowan 115* (holotype: US!).

A var. *ovifera* atque var. *texana* allele *m* ad locu *Idh-2* e isocitrate dehydrogenase et germinatione praecoci seminum, a var. *texana* fructibus eburneis differt. (Author's translation: Is distinguished from var. *ovifera* and var. *tenana* by allele *m* at locus *Idh-2* of isocitrate dehydrogenase and early seed germination, is distinguished from var. *texana* by having ivory fruits).

Morphology. (Only fruits and seeds have been studied in any detail [see Smith et al. 1992; Cowan and Smith, this volume].) Mature fruits of *C. pepo* ssp. *ovifera* var. *ozarkana* are bitter, ivory or rarely green- and white-striped, oblate to round to prolate to pyriform, and measure 3.2–4.6 cm in diameter and 3.9–10.0 cm in height. Rinds are thin, measuring 0.8–2.2 mm in thickness, and smooth. Peduncles measure 5.3–8.6 mm in diameter at their base. Seeds are 7.8–10.1 mm long and 5.2–6.7 mm wide. Deviations from these character states have been interpreted as evidence of cultivar introgression (Cowan and Smith, this volume).

Isozymes. This variety is distinguished from other varieties of ssp. *ovifera* by possession of the isozyme allele *Idh-2m*. Populations of var. *ozarkana* are further characterized by the alleles *Aat-1d* and/or *-1e⁻, - 3j, -4r* and/or *-4u; G2d-1k, -2v; Gpi-3u; Idh-1e⁻, -2g, -3m; Lap-1f; Mdh-2e* and/or *-2i, -3m* and/or *-3q; Pgm-5o, -6v;*

and *Skd-1i* and/or *-1m*. *Aat-3v*$^{-}$, *G2d-1i, Idh-3o,* and *Lap-1f*$^{n}$ are relatively rare alleles found in some populations.

Distribution and ecology. Populations of var. *ozarkana* have been discovered in western Illinois, southern Missouri, Arkansas, eastern Oklahoma, and Louisiana. This taxon's distribution may include similar populations in Kentucky and Alabama. Populations appear most abundant along rivers and streams draining the Ozark Plateau and the Ouachita Mountains. The plants are adapted to the naturally disturbed areas in riverine floodplains and can invade humanly disturbed habitats as well. Fruits are water-dispersed. This variety is distinguished from other varieties of ssp. *ovifera* by the relatively quick seed germination (usually within two days). The distribution and ecology of var. *ozarkana* are described more fully in Smith et al. (1992).

Deviating most from the typical var. *ozarkana* isozyme and morphological profiles were populations at the northern and eastern extremes of the range as we know it, in Kentucky, Illinois, and Alabama. Of these, the most likely candidate for a purely feral population was that represented by the fruit from Kentucky. We consider the evolutionary status of the Alabama populations to be uncertain.

*Evolutionary trends in C. pepo.*—Because previous genetic work (Decker 1988; Wilson et al. 1992) had already shown that cultivars of ssp. *ovifera* and ssp. *pepo* were derived from genetically divergent population lineages, it was relatively easy to detect and assess as introgressants in wild populations of ssp. *ovifera* those alleles that were otherwise restricted to and characterized ssp. *pepo*. First-generation gene flow from cultivars to wild populations was detected in several populations of var. *ozarkana* and in at least one population of var. *texana* (Decker and Wilson 1987). Although recent introgression is suggested by various other populations, the significance and temporal depth of hybridization between cultivars and wild populations is unclear. Obviously, introgression into the wild has not occurred to the extent of obliterating a more ancient genetic background in var. *ozarkana*.

Evolutionary relationships among taxa of *C. pepo* are indicated by the isozyme data. Most, if not all, primitive cultivars of var. *ovifera* apparently have their origins in var. *ozarkana*. Variety *ozarkana* possesses the isozyme patterns typical of a wild progenitor of a crop: isozyme alleles in the crop (i.e., var. *ovifera*) are generally a subset of those in the progenitor (i.e., var. *ozarkana*) and the two taxa are genetically similar, usually with a genetic identity value of 0.90 or greater (cf. Doebley 1989). The fact that Doebley's (1989) extensive survey of isozyme variation in crops and their putative progenitors yielded the "subset" criterion says the following about the effects of domestication on isozyme variation: few, if any, new alleles are introduced as a result of human intervention. This is why it was concluded previously (i.e., Decker 1988) that domesticated members of ssp. *pepo* and ssp. *ovifera* were selected from genetically diverged wild populations already exhibiting the distinct alleles found in these subspecies today.

Although ssp. *fraterna* has a genetic identity value with ssp. *ovifera* var. *ovifera* that is also close to 0.90, ssp. *fraterna* apparently lacks at least three alleles (*Mdh-2e, Mdh-3m, Skd-1m*) that are in high frequencies in cultivars of ssp. *ovifera;* hence ssp. *fraterna* fails the first criterion wherein alleles of the crop are a subset of those in the

wild progenitor. Nevertheless, the principal component analyses did indicate close overall genetic affinities between a few cultivars that have been classified under ssp. *ovifera* var. *ovifera* (i.e., 'Flat Striped') and ssp. *pepo* (i.e., 'Orange Ball'), indicating that at least some cultivars may have been selected from populations in northeastern Mexico (cf. Andres 1987). Further study of ssp. *fraterna* is needed to make these determinations.

Most analyses revealed a relatively distinct isozyme pattern for ssp. *ovifera* var. *texana*. Like ssp. *fraterna*, Texas populations lack various alleles common in ssp. *ovifera* var. *ovifera*. In addition, almost all populations of var. *texana* contain an allele (*Idh-3o*) not found in any cultivars. Consequently, evidence is lacking which would suggest that any cultivar or group of cultivars have their direct origins in Texas populations.

Applying Doebley's (1989) criteria in search of the wild ancestor of Mexican landraces of ssp. *pepo*, we do not find a good candidate for progenitor among currently known wild populations. Consequently, we believe that populations of this wild progenitor are undiscovered or probably extinct. Furthermore, the pat tern of genetic differentiation (as discussed below) between ssp. *pepo* and the other taxa, including the Mexican ssp. *fraterna*, suggests that the progenitor of ssp. *pepo* evolved in a distinct area of Mexico.

Examination of *C. argyrosperma* ssp. *sororia* gave us an additional evolutionary perspective on isozyme variation of *C. pepo*. The fact that *C. argyrosperma* ssp. *sororia* exhibited more or less equally divergent but genetically distinct relationships with each taxon of *C. pepo* indicates that long periods of reproductive isolation due to geographic separation probably account for differences among ssp. *ovifera* var. *ozarkana*, var. *texana*, ssp. *fraterna*, and the hypothesized progenitor of ssp. *pepo*. Such a conclusion fits Wiley's model of vicariance speciation in which geographically disjunct progenitor populations are replaced by more divergent and genetically different populations through time (Wiley 1981; Wiley and Mayden 1985). This means that none of the wild populations known today is genetically equivalent to what were the most ancient populations of *C. pepo*. This interpretation of geographic divergence requires fewer *ad hoc* assumptions than in presuming that a mixture of other factors (e.g., selection) produced this pattern of uniform divergence among taxa of *C. pepo*. Of course, what requires the fewest assumptions does not necessarily reflect reality and factors such as human and natural selection (e.g., seed germination rates) and population bottlenecks (e.g., relative genetic homogeneity in ssp. *ovifera* var. *texana*) have undoubtedly contributed to the genetic patterns we see today. With those possibilities in mind, we speculate that the original distribution of wild populations of *Cucurbita pepo*, which ranged from central or southern Mexico north, at least to the Ozark Plateau, and east, possibly as far as Florida (Decker and Newsom 1988; Newsom et al., this volume), became dissected long before at least two independent domestications took place.

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